

REMARKS

In response to the election requirement, the Applicants withdraw without prejudice claims 3, 10, 19-42, 45, 46 and 48 to 51 and submit the listing of claims enclosed herewith in replacement of all prior versions. Claim 6 is rewritten to reflect correct amendments to the claim.

In this new listing of the claims:

Claims 1, 2, 6 and 7 have been amended;

Claim 8 is original;

Claim 9 has been amended;

Claim 43 has been amended;

Claim 47 is original;

Claims 53 to 57 are new and find proper support in various sections of the specification;

Claims 58 and 59 are new and correspond respectively to claims 12 and 18 presently on file; and

Claims 4, 5, 11 to 18 and 44 presently on file have been withdrawn without prejudice.

As requested by the Examiner in **paragraph 4**, a new and corrected Declaration duly signed by GIDDA SATINDER is enclosed.

In **paragraph 5**, The Examiner noted that JP 02-092220 listed in the IDS filed by the Applicants has not been provided. Accordingly, a copy of the abstract of this publication in English, and a copy of the publication in Japanese are provided herewith for the Examiner's convenience.

In **paragraph 6**, the Examiner has rejected claims 6, 14 and 44 presently on file for reading on non-elected inventions. In response to this rejection, the Examiner will note that the new set of claims has been limited to the elected invention.

In **paragraph 7**, the Examiner has objected to the specification because of the embedded hyperlinks. In response to this rejection the hyperlinks appearing on pages 17 and 18 of the specification have been deleted.

In **paragraph 8**, The Examiner has rejected claims 6 to 8 and 14 presently on file under 35 U. S. C. 112, second paragraph as being indefinite and failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. In response to this rejection, the new claims now refer to a SEQ ID from the sequence listing.

In **paragraphs 9 and 10**, the Examiner has rejected claims 1, 2, 4 to 9, 11 to 18, 43, 44 and 47 presently on file under 35 U.S.C. 112 first paragraph as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention, and because the specification is only enabling for a method of increasing time to flowering in *Arabidopsis* plants comprising transforming said plants with the *Arabidopsis* AtST2a genomic sequence of SEQ ID NO:1, operably linked to a promoter in antisense orientation, wherein the level of 12- or 11- hydroxyjasmonic acid are increased relative to non-transgenic plants.

In response to this rejection, the Applicants submit a new listing of the claims relating to a method of increasing time to flowering in *Arabidopsis* plants, comprising transforming said plants with the *Arabidopsis* AtST2a genomic sequence of SEQ ID NO:1, operably linked to a promoter in antisense orientation, wherein the level of 12- or 11- hydroxyjasmonic acid are increased relative to non-transgenic plants. Hence, the listing of claims is now fully supported and enabled by the description.

Moreover, the sequence described in the present application contains the motifs that are well known to be present in all soluble sulfotransferases that have been characterized so far. It is possible to find this information by doing a protein blast search at NCBI. More specifically, two domains are highly conserved.

- The first one comprises the sequence YPKSGTTW and is localized at the N-terminal of all sulfotransferases. The lysine residue of this domain has been shown to bind the sulfate donor 3'-phosphoadenosine 5'-phosphosulfate (see Marsolais, F and Varin, L. (1995) Identification of amino acid residues critical for catalysis and substrate binding in the flavonol 3-sulfotransferase. *J. Biol. Chem.*, **270**, 30458-30463.)
- The second domain, RKXXGDWKNXFT, is localized closer to the C-terminal extremity. The arginin residue of this motif is critical for the binding of the sulfate donor.
- There are also other amino acid residues that have been shown to be absolutely required for activity such as histidine 118 (numbering of the flavonol 3-sulfotransferase) which acts as base catalyst during catalysis.

It is very easy for a person skilled in the art, namely, one having expertise in protein chemistry, to find these conserved domains and to assess the sulfotransferase function to an unknown protein having these motifs.

Another reference that can be used to find the structural characteristics of sulfotransferases is: Marsolais, F. and Varin, L. (1998) Recent developments in the study of the structure-function relationship of flavonol sulfotransferases. *Chem. Biol. Interact.*, **109**, 117-122.

The Applicants also submit that knowing the conserved domains present in all soluble sulfotransferases, it is very easy to identify in databases protein sequences having sulfotransferase activity. A FASTA search in SWISSPROT using the string YPKSGTTW will retrieve a large number of sequences that have already been characterized at the biochemical level or that are predicted to encode sulfotransferases.

To search for new sulfotransferase coding sequences, a PCR approach using partially degenerate oligonucleotides targeting the two conserved domains would allow amplification of DNA fragments encompassing a large portion of sulfotransferase coding sequences. The PCR products can then be used to screen libraries to search for full-length clones. Someone skilled in the art can easily perform this molecular approach.

Finally, despite the fact that it is difficult to predict exactly the outcome of plant transformation experiments due to the random insertion of the T-DNA in transgenic plants, the characterization of a number of independent lines allow to find the one(s) expressing the transgene at an adequate level. This is true both for overexpression in sense and underexpression via antisense or RNA interference. Someone skilled in the art, would easily bypass this apparent pitfall of transgenic plant production.

In **paragraph 11**, the Examiner has rejected claims 1 and 2 presently on file under U. S. C. 102(b) as being anticipated by Krajncic et al. (1995, J. Plant Physiol. 146:754-756). In response to this rejection, claims 1 and 2 have been amended.

Hence new claims 1 and 2 are now directed to a method for modulating or inducing flowering in a plant, comprising modifying in said plant the endogenous level of at least one compound selected from the group consisting of 12-hydroxyjasmonic acid, glucoside of 12-hydroxyjasmonic acid, sulfate ester of 12-hydroxyjasmonic acid, 12-hydroxymethyljasmonic acid, glucoside of 12-hydroxymethyljasmonic acid, sulfate ester of 12-hydroxymethyljasmonic

acid, 11-hydroxyjasmonic acid, glucoside of 11-hydroxyjasmonic acid, sulfate ester of 11-hydroxyjasmonic acid, 11-hydroxymethyljasmonic acid, glucoside of 11-hydroxymethyljasmonic acid, sulfate ester of 11-hydroxymethyljasmonic acid, and mixtures thereof, wherein the endogenous level of at least one compound is modified by modulating the expression of a sulfotransferase encoded by a gene of SEQ ID NO:1.

On the other hand, the scientific article by Krajncic et al. discloses the addition of jasmonic acid solution to the nutrient solution of the experimental plants *Spirodela polyrrhiza* and does not disclose the modulation of the expression of a sulfotransferase encoded by a gene of SEQ ID NO:1.

Moreover, and as described in the application, the novelty of the present invention resides in the fact that the Applicants has demonstrated that it is not jasmonic acid which is responsible for the induction of flowering, but 12-hydroxyjasmonate. The Applicants agree that the treatment of plants with jasmonic acid can affect flowering time but submits that it will also affect other aspects of plant development. The Applicants also agree that a mutation in the jasmonate pathway will ultimately lead to the absence of 12-hydroxyjasmonate and consequently give rise to late flowering plants, but the present invention has the advantage to keep the jasmonate pathway intact. For example, a knock-out mutation of the gene encoding the enzyme Allene Oxide Synthase (AOS) results in plants which are deficient in jasmonic acid (J-H Park et al. 2002) but as stated in the research article, these plants are also defective in wound signal transduction and are more susceptible to pathogen infection.

Similar negative side effects were obtained with plants having a mutation in the genes encoding the enzyme Allene Oxide Cyclase (AOC), 12-oxo-phytodienoic acid reductase (OPR3) fatty acid desaturase (FAD3, 7 and 8) and lipoxygenase 2 (LOX2).

It has been demonstrated that jasmonic acid as well as its precursor 12-oxo-phytodienoic acid (OPDA) regulate the expression of several genes involved in the plant defense response. In addition, it has been demonstrated that the application of jasmonic acid to plants has deleterious effects on growth (such as repression of genes involved in photosynthesis, root growth inhibition and loss of chlorophyll). Similar experiments conducted with 12-hydroxyjasmonate did not give rise to similar negative side effects. This can be explained by the fact that 12-hydroxyjasmonate does not induce or repress the same genes when compared with jasmonic acid or OPDA.

To illustrate the difference between the effect mediated by jasmonic acid and 12-hydroxyjasmonate on gene expression, selected results from an mRNA profiling experiment performed with the *A. thaliana* Affymetrix DNA chips are presented in Table 1, 2 and 3. It is important to note that only selected genes are presented and that these results have not been published. The Affymetrix DNA chips comprise more than 22,000 entries and a large number of genes are clustering with the ones presented in the three Tables.

The results show clearly that jasmonic acid and 12-hydroxyjasmonate have different effects on gene expression. For example, 12-hydroxyjasmonate does not repress the expression of genes involved in photosynthesis (Table 1). Furthermore, 12-hydroxyjasmonate does not induce the expression of *THI2.1* a marker gene in the plant defense response (Table 2). A similar result with *THI 2.1* was presented in the publication by Gidda, S *et al.* (2003) *J. Biol. Chem.* 278, 17895-17900.

The microarray results clearly show that 12-hydroxyjasmonate induces gene expression in *A. thaliana* and that this induction is independent of the jasmonic acid induction pathway (Table 3).

To summarize, the advantages of modulating the endogenous levels of 12-hydroxyjasmonate by sulfonation over knocking down the synthesis of jasmonic acid to control flowering time are:

- The absence of negative side effects on growth.
- The absence of negative side effects on the defense response.

Hence, the Applicants submit that new claims 1 and 2 are new over the cited prior art and the Examiner is kindly requested to reconsider his rejection under U. S. C. 102(b).

In view of the above arguments and amendments, the Application is believed to be in condition for allowance.

Table 1. Selected genes encoding enzymes involved in photosynthesis.

Accession	Control	methyl jasmonate*	12- hydroxyjasmonate**
At1g44446	4164***	555	3810
At5g01530	42663	29136	45635
At4g27440	19087	4315	21581
At1g29910	48449	27488	53212
At1g19150	9037	6366	9204

* Plants were treated with 50 micromolar of methyl jasmonate for a period of four hours.

** Plants were treated with 50 micromolar of 12-hydroxyjasmonate for a period of four hours.

*** Numbers represent normalized gene expression values. Three independent biological replicates were used for this study.

Table 2. Selected genes encoding proteins involved in the plant defense response

Accession	Control	methyl jasmonate	12- hydroxyjasmonate
At5g07010	120	9744	223
At3g55970	130	6956	90
At1g63040	8	296	8
At1g72260	104	3487	64
At1g54020	71	2278	59
At5g42900	73	1905	69
At3g16330	16	363	16
At3g20810	198	3950	168
At5g37260	56	1105	50
At2g22880	8	148	9
At3g23550	247	4029	250

* Plants were treated with 50 micromolar of methyl jasmonate for a period of four hours.

** Plants were treated with 50 micromolar of 12-hydroxyjasmonate for a period of four hours.

*** Numbers represent normalized gene expression values. Three independent biological replicates were used for this study.

Table 3. Selected genes induced by 12-hydroxyjasmonate

Accession	Control	methyl jasmonate	12- hydroxyjasmonate
At1g30140	3	3	44
At4g04070	4	7	73
At5g21110	4	3	72

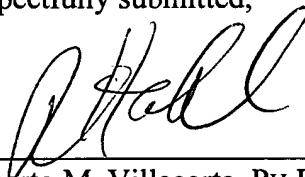
* Plants were treated with 50 micromolar of methyl jasmonate for a period of four hours.

** Plants were treated with 50 micromolar of 12-hydroxyjasmonate for a period of four hours.

*** Numbers represent normalized gene expression values. Three independent biological replicates were used for this study.

Applicants' undersigned attorney may be reached in our Washington, D.C. office by telephone at (202) 625-3500. All correspondence should continue to be directed to our address given below.

Respectfully submitted,



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Date: May 19, 2005

Attachments: Declaration
Japanese Publication No. 02-092220
Copy of USPTO date-stamped receipt

Patent Administrator
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DECLARATION FOR PATENT APPLICATION

As a below named inventor, I (we) hereby declare that my (our) residence, post office address and citizenship are as stated below next to my (our) name; I (we) believe that I am (we are) the original, first and sole inventor(s) (If only one name is listed below) or an original, first and joint inventor (If plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention (Design, if applicable) entitled: METHODS, COMPOSITIONS AND GENETIC SEQUENCES FOR MODULATING FLOWERING IN PLANTS AND PLANTS GENETICALLY MODIFIED TO FLOWER EARLY AND LATELY

the specification of which (check one): ☐ is attached hereto; ☒ was filed on May 19th, 2002 as application serial No. 10/018,831 and was amended on (or amended through) JULY 28th, 2004 (if applicable); ☐ was filed on _____ as International Application (PCT) No. _____ and amended on _____ (if applicable). I (we) hereby state that I (we) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I (we) acknowledge the duty to disclose information known by me (us) to be material to the patentability of my (our) invention in accordance with Title 37, Code of Federal Regulations, § 1.56(a). I (we) hereby claim foreign priority benefits under Title 35, United States Code § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application which priority is claimed.

I (We) hereby claim foreign priority benefits under Title 35, United States Code § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application which priority is claimed.

Prior Foreign Application(s)

(Number)	(Country)	(Day/Month/Year Filed)
<u>2,274,873</u>	<u>CANADA</u>	<u>08/07/1998</u>
_____	_____	_____
_____	_____	_____

Priority Claimed

<input checked="" type="checkbox"/> YES	<input type="checkbox"/> NO
<input type="checkbox"/> YES	<input type="checkbox"/> NO

I (we) hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code § 112, I (we) acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior art application and the national or PCT international filing date of this application:

(Appl. No.)	(Filing date)	(Status - Patented, Pending or Abandoned)
_____	_____	_____
_____	_____	_____

I (we) hereby declare that all statements made herein of my (our) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I (we) hereby appoint the attorneys associated with the following customer number, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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PATENT ABSTRACTS OF JAPAN

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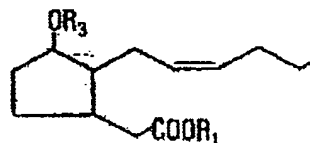
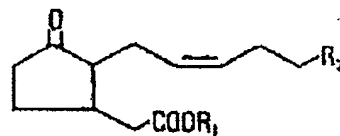
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(54) POTATO TUBER FORMING AND INDUCING AGENT AND METHOD FOR FORMING AND INDUCING POTATO TUBER

(57)Abstract:

PURPOSE: To surely form and induce large amounts of potato tuber by adding ascorbic acid and jasmonic acid compounds to a culture medium.

CONSTITUTION: A stem fragment containing a terminal bud or nod reared by shoot tip culture or rooting transfer method of potato plant is reared in tissue culture medium (e.g. Linsmaier & Skoog) for about 4 weeks to provide an aseptic shoot. 10-5000ppm ascorbic acid and 0.3-12ppm jasmonic acid compound expressed by formula I or formula II (R₁ and R₂ are H or 1-10C alkyl; R₂ is H, OH, O-D-glucopyranose) and as necessary 0.5-10ppm cytokinins compound (e.g., kinetin) used as a potato tuber-forming and inducing agent are added to a culture medium containing the above-mentioned aseptic shoot and the shoot is cultured for 2-4 weeks to form potato tuber at the nod of aseptic shoot.

**LEGAL STATUS**

[Date of request for examination]

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⑤ 日本国特許庁(JP) ⑥ 特許出願公開
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⑧ Int.Cl.⁹ 識別記号 片内整理番号 ⑨ 公開 平成2年(1990)4月3日
 A 01 H 4/00 8502-2B
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 8515-4B C 12 N 5/00 F ※
 審査請求 未請求 請求項の数 5 (全4頁)

⑩ 発明の名称 馬鈴薯塊茎形成誘導剤及び同形成誘導方法

⑪ 特 願 昭63-242432

⑫ 出 願 昭63(1988)9月29日

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最終頁に続く

項 目 名

1. 発明の名称

馬鈴薯塊茎形成誘導剤及び同形成誘導方法

2. 特許請求の範囲

(1) アスコルビン酸とジャスモン酸誘導化合物とを有効成分として含有することを特徴とする馬鈴薯塊茎形成誘導剤。

(2) ジャスモン酸誘導化合物が12-8-0-D-グルコピラノシロキジャスモン酸、メチルジャスモン酸、ジャスモン酸又は6-ヒドロキレジャスモン酸である請求項1の馬鈴薯塊茎形成誘導剤。

(3) サイトカイニン誘導化合物をも有効成分として含有する請求項1又は2の馬鈴薯塊茎形成誘導剤。

(4) サイトカイニン誘導化合物がカイネチンである請求項3の馬鈴薯塊茎形成誘導剤。

(5) 組織培養培地中に請求項1、2、3又は4の馬鈴薯塊茎形成誘導剤を添加することを特徴とする馬鈴薯塊茎形成誘導方法。

3. 発明の詳細な説明

(産業上の利用分野)

本発明は、馬鈴薯塊茎形成誘導剤及び同形成誘導方法に関する。特に、組織培養方法を用いて馬鈴薯塊茎形成誘導する際に有用な馬鈴薯塊茎形成誘導剤及び同形成誘導方法に関する。

(従来の技術)

従来、馬鈴薯の組織培養によって得られる塊茎を馬鈴薯の無菌的繁殖増殖方法に用いることが注目されている。この方法においては、馬鈴薯塊茎を組織培養して、塊茎を形成誘導する点にポイントがある。

塊茎を形成誘導するのに用いる組織培養培地の組成が、「国研学会昭62年秋大会誌」第2巻、227頁(記載者、秋田 実、高山貞實)において、既に提案されている。

同刊行物では、まず、組織培養培地であるムラゼンスクーグ(Murasei-Skoog)培地のシェーククロス濃度を3%に調整した培地で組織培養して、無菌シュートを育成(Phase 1)し、次に、

特開平2-82220 (2)

同培地のシェーククロス濃度を高濃度(8%)に調整した培地で組織培養(Phase 2)して、塊莖の形成率を増大させたことが報告されている。

この方法では、Phase 1で育成された無菌シュートにPhase 2の培地に移植するか、Phase 2の培地に取り替えることを必要とし、この際、多大の労力を要する点に課題があった。

(発明が解決しようとする課題)

本発明は、従来技術に見られる上記課題を解決するとともに、一層有効な塊莖形成誘導剤及び同剤を用いた塊莖形成誘導方法を提供せんとするものである。

(課題を解決するための手段)及び(作用)

本発明は、アスコルビン酸とジャスモン酸誘導化合物とを有効成分として含有することを特徴とする塊莖形成誘導剤、アスコルビン酸とジャスモン酸誘導化合物とサイトカイニン誘導化合物とを有効成分として含有することを特徴とする塊莖形成誘導剤及び前記二剤のいずれかを組織培養培地中に添加することを特徴とする塊莖形成誘導方法。

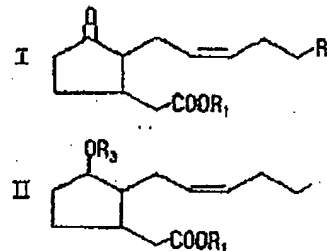
モン酸、メチルジャスモン酸、ジャスモン酸又は6-ヒドロキシジャスモン酸である。

本発明に用いられるサイトカイニン誘導化合物とは、カイネチン、ゼニルアミノプリン、フェニルアミノプリン、ベンジルアミノプリン、シクロヘキシルアミノプリン、オータクロベンジルアミノプリン、ロータシルベンジルアミノプリン、ジフェニルアミン、4-ピリジルフェニル炭素、4-ベンジルアミノベンズイミダゾール、6-イソペンタニルアミノプリン、トランスセアチン、シスセアチン、トランスセアチンリボシド、トランスセアチンモノリボシド、ジヒドロセアチンなどである。

塊莖形成を形成誘導するためには、まず、低細胞物の誘導点培養又は塊根移植により育成した頂芽又は節を含む茎断片(以下、これを「断片」という。)を組織培養培地で約4週間育成して、無菌シュートを得る。次に、無菌シュートの培地中に、アスコルビン酸100~5000ppm、好ましくは500~2000ppmとジャスモン酸誘導化合物0.3~12ppm、

形成誘導方法を要旨とするものである。

本発明に用いられるジャスモン酸誘導化合物とは、次の一般式I又はIIで表される化合物である。



(なお、上記一般式中
R₁、R₂は、H又はCが1~10のアルキル基
R₃は、H、OH又はα-β-D-グルコピラノースを示す。

上記ジャスモン酸誘導化合物は、好ましくは、1,2-β-D-グルコピラノシロキシジャス

モン酸又は1~5ppmとを添加し、さらに2~4週間培養すると無菌シュートの節に塊莖が形成誘導されるのである。

同様にして、無菌シュートの培地中に、アスコルビン酸100~5000ppm、好ましくは500~2000ppmとジャスモン酸誘導化合物0.3~12ppm、好ましくは1~5ppmとサイトカイニン誘導化合物0.5~10ppm、好ましくは1~5ppmを添加し、さらに2~4週間培養すると無菌シュートの節に塊莖が形成誘導されるのである。

(実施例)

塊莖形成断片を組織培養する培地として、第1表に示す組成を有するリンスマイヤー・スコーグ(Linsmaier & Skoog)培地(以下、「L S培地」と略称する。)を用いた。

第1表 L S培地組成 (mg/l)

HgSO ₄ ·7H ₂ O	370	CeCl ₃ ·2H ₂ O	440
KNO ₃	1,900	HN ₃ NO ₂	1,650
KH ₂ PO ₄	170	FeSO ₄ ·7H ₂ O	27.8
H ₂ EDTA	37.3	HnSO ₄ ·4H ₂ O	22.3

特開平2-92220(公)

$ZnSO_4 \cdot 7H_2O$	0.4	$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025	KI	0.83
H_2BO_3	6.2	$Na_2MoO_4 \cdot 5H_2O$	0.25
水-28-8	30,000	水-1/30-8	100
硫酸ナトリウム	0.4		

組織培養は、直径2.2cm、高さ1.5cmの管ビン中に1.5gの培地1.0mlを入れ、20℃連続培養条件下で4週間培養し、平均直径1.2cmの無菌シュートを育成した。無菌シュートを切断して得た切片を、さらに、同様の条件下で培養を繰り返して、供試無菌シュートを必要数育成した。

このようにして得た無菌シュートの管ビン中に、予め第2表に示す組成に調整した水溶液を100μlを添加した。さらに、20℃連続培養条件下で2週間後及び4週間後に培養の形成度を調べた。

(以下 表)

第3表 培養法の形成度

	2週間後	4週間後
本発明区1	2.1	3.2
本発明区2	1.7	2.6
本発明区3	2.0	2.7
本発明区4	2.1	3.2
本発明区5	2.0	3.1
単独使用区		
7-アスコルビン酸:1,000ppm	0	0.3
12-β-D-グルコピラノシロキシジヤスモン酸		
3-アスコルビン酸:3.88ppm	0	0
メチルジヤスモン酸:2.24ppm	0	0.2
3-アスコルビン酸:2.10ppm	0	0
6-ヒドロキシジヤスモン酸:2.12ppm	0	0
4-ヒドロキシジヤスモン酸:2.5ppm	0	0.1
従来区	1.8	2.3

注) 1. 形成度は、いずれも10回反復の平均値。

2. 本発明区の培養には、いずれもアスコルビン酸1000ppmを含有するとともに、

第2表 調整した水溶液の組成(100μl中)

	7-アスコルビン酸	3-アスコルビン酸化合物 (化合物名: 添加量)	4-アスコルビン酸
本発明区1	10mg	12-β-D-グルコピラノシロキシジヤスモン酸: 3.88μg	0μg
本発明区2	10mg	メチルジヤスモン酸: 2.24μg	0μg
本発明区3	10mg	3-アスコルビン酸: 2.10μg	0μg
本発明区4	10mg	6-ヒドロキシジヤスモン酸: 2.12μg	0μg
本発明区5	10mg	4-ヒドロキシジヤスモン酸: 2.5μg	25μg

対照区は、L-アスコルビン酸、12-β-D-グルコピラノシロキシジヤスモン酸、メチルジヤスモン酸、ジヤスモン酸、6-ヒドロキシジヤスモン酸及びカイネチンをそれぞれ単独に、同様にして添加した単独使用区並びにシュクロース濃度を9%に調整した1.5g培地は、無菌シュートを育成した従来区とし、組織培養条件は、いずれも同一とした。

その結果を第3表に示す。

本発明区1には、12-β-D-グルコピラノシロキシジヤスモン酸3.88ppm、本発明区2には、メチルジヤスモン酸2.24ppm、本発明区3には、ジヤスモン酸2.10ppm、本発明区4には、6-ヒドロキシジヤスモン酸2.12ppm、本発明区5には、12-β-D-グルコピラノシロキシジヤスモン酸3.88ppm及びカイネチン2.5ppmを含有する。

第2表から明らかな通り、本発明区1～5は、いずれも2及び4週間後の培養形成度で、従来区を上回り、優れた培養形成度があることを示した。単独使用区は、いずれも培養ほとんど形成しなかった。

(尚書)

本発明の高純度培養形成誘導剤及び果物培養誘導剤の形成誘導方法によって、果物培養物の組織培養によって、大量の培養を確実に形成誘導することができる。

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特開平2-92220(4)

第1頁の続き

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